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In Vitro Biocompatibility of Red Tomato Cells (*Solanum lycopersicum*) and Green Tomato Cells (*Physalis philadelphica*) over hydrogels based on collagen-starch-Mo-MOFs

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ABSTRACT

The germination and growth of red tomato *(Solanum lycopersicum)* and green tomato *(Physalis philadelphica)* plants depends on water availability and micronutrients from the soil. Then, hydrogels based on collagen, starch, and Mo-MOFs were synthesized to provide enough water and micronutrients such as molybdenum and amino acids (histidine, phenylalanine, tryptophan), suitable to allow the growth, proliferation, and migration of red tomato and green tomato cells living over these hydrogels. FTIR and ninhydrin essay studied the main functional groups of hydrogels and their reticulation index, respectively. Also, the MTT test served to measure the metabolism of red tomato and green tomato cells (extracted from seeds) living on these hydrogel surfaces. At the same time, the cell migration of red tomato and green tomato cells was observed when cells were encapsulated into the hydrogels and observed by microscope. The main results show that the hydrogel with Mo-MOF composed of tryptophan presents the best cell viability and cell migration essay, this is indicative that tryptophan is non-cytotoxic and does not disturb the metabolism of vegetal cells, but also, that within this microenvironment vegetal cells from red and green tomato are allowed to form vegetal tissues, and thus germinate quickly in a controlled environment.

Keywords: Red tomato *(Solanum lycopersicum)*; Green tomato *(Physalis philadelphica)*; Hydrogel; Collagen; Starch; Tryptophan; Phenylalanine; Molybdenum; MOFs; Histidine.

1. Introduction

Mexico is a country where important worldwide crops such as beans, cacao, cotton, maize, papaya and tomatoes have been originated and diversified [1]. In the case of red tomato, México supplies the 1.70% of the world tomato production which represents 19% of the volume of exports worldwide, this places it above Spain (14%), and the Netherlands (13%) [2].

Nitrogen is crucial for tomato production because the high yield and fruit quality depend on it. However, a high application of fertilizers with nitrogen can reduce the uptake efficiency and increase nitrogen leaching and post-harvest soil nitrogen residues. Also, soil irrigation is important for maintaining nutrients in the crop root zone, and thus, is challenging in the case of sandy soils [3].

A promising solution to provide enough water and micronutrients to tomato crops is the application of hydrogels based on biopolymers to avoid the damage that is caused by conventional fertilizers such as pollution of air, water, and soil; as well as the destruction of soil fertility in the long term, and the spread of cancer agents [4]. In this manner, hydrogels have been applied in agriculture as water and nutrient reservoirs allowing plants to grow in arid zones, and seeds to be germinated; without the need for herbicides, or fertilizers, and reducing the frequency of irrigation. Hydrogels chemically composed of cellulose, chitosan, starch, lignin, and kenaf fiber are biodegradable, low-cost, and non-toxic; they are suitable for the growth of crops such as tomatoes [5].

Our research group has published many articles related to hydrogels based on collagen and polysaccharides such as starch [6,7]. Indeed, in a recent article, it was exposed that a low starch content (10-20 wt.%) in the collagen matrix

enhances the hydrophilic and mechanical properties of hydrogels without compromising their biodegradability [8]. In addition, in some recent articles, we have published the chemical and biological characterization of MOFs based on biocompatible metals such as Zn, Ca, and Mo using as organic ligands amino acids like histidine, tryptophan, and phenylalanine, that have been incorporated into collagen-guar gum hydrogel biomatrix. The *in vitro* biocompatibility of these hydrogels has been proved satisfactorily in the metabolism of porcine fibroblasts [9-11].

MOFs based on molybdenum and amino acids like histidine, phenylalanine, and tryptophan have been incorporated into the hydrogel matrix of collagen and starch (Figure 1). The idea was to test the *in vitro* biocompatibility by measuring the metabolism of red tomato and green tomato cells extracted from seeds when they are living on the hydrogel surface. As well as the cell migration of red and green tomato cells observed by microscope when cells are encapsulated into the hydrogel matrix. In addition, the hydrogels were characterized by ATR-FTIR, and the reticulation index was obtained to correlate these chemical properties with the *in vitro* biocompatibility.

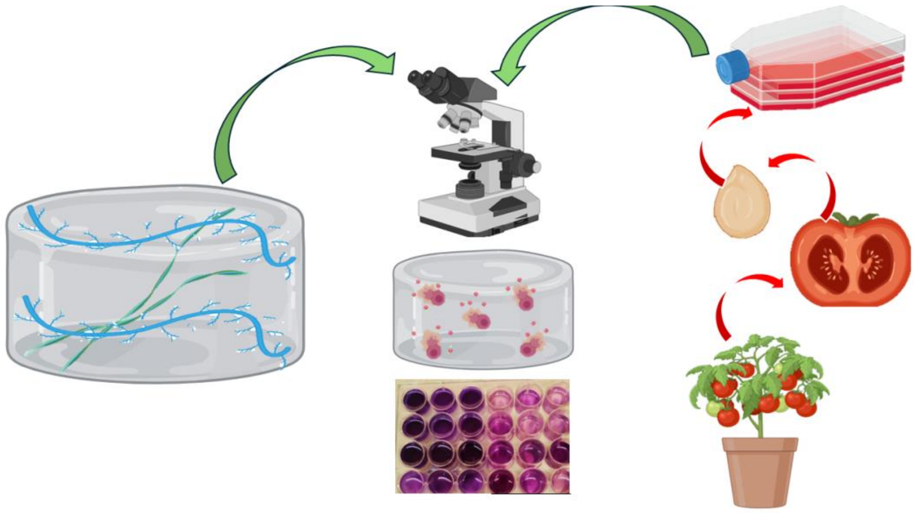

Figure 1. Design of collagen-starch-MoMOFs hydrogels for *in vitro* biocompatibility of red and green tomato cells

1.1. Study objectives

The following are the main objectives of this study. (i) The synthesis of composite hydrogels based on collagen, with a network modified with starch and Mo-MOFs composed of amino acids: histidine, phenylalanine, and tryptophan. (ii) Study of the functional groups present in composite hydrogels by ATR-FTIR to corroborate the physicochemical interactions of all the components. (iii) Analyzed the reticulation index of hydrogels to elucidate if Mo-MOFs form physical or chemical interactions. (iv) Study the effect of the amino acid type in the *in vitro* biocompatibility in red and green tomato seed cells. (v) Investigate the repercussions of the amino acid in the cell migration of red and green tomato seed cells.

2. Materials and Methods

2.1. Chemicals

L-histidine (His), L-phenylalanine (Phe), L-tryptophan (Try), ammonium heptamolybdate ($(NH_4)_6Mo_7O_{24}$•$4H_2O$), 1,3,5-benzenetricarboxylic acid (TMA), starch (soluble, extracted form potato, Mn 300 g mol^{-1}), 3-(4,5-Dimethyl-

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2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) and Murashige and Skoog (MS) culture were purchased from Sigma Aldrich Co., and they were used as received. Collagen type I was extracted from porcine dermis was extracted by enzymatic hydrolysis with pepsin as reported elsewhere (Mn α_1 = 220 000 g mol^{-1}, α_2= 110 000 300 g mol^{-1}) [12]. The polyurethane crosslinker (Mn 3000 to 7500 g mol^{-1}) was elaborated from glycerol ethoxylate and 1,6-hexamethylene diisocyanate and glycerol ethoxylate using the procedure reported elsewhere [13]. Red tomato (*Solanum lycopersicum*) and green tomato (*Physalis philadelphica*) seeds were bought from a local greenhouse.

2.2. Synthesis of Mo-MOFs

Molybdenum-amino acid MOFs were synthesized by the hydrothermal method. In a typical synthesis procedure water solutions of 1 mmol of ammonium heptamolybdate, 1 mmol of TMA, and 1 mmol of the suitable amino acid (His, Phe, or Try) were mixed with magnetic stirring at room temperature adjusting its pH to 4.4 [11]. Then, the mixture was transferred to a Teflon-lined autoclave, and the reaction was carried out at 120 °C for 72 h. The white precipitate obtained from the reaction was filtered, rinsed with water, and dried at 60 °C. The obtained molybdenum coordination polymers were labeled as MoHis, MoPhe, and MoTry depending on the amino acid used as ligand.

2.3. Synthesis of collagen-starch-Mo-MOFs hydrogels

The hydrogels based on collagen, starch and molybdenum-amino acid MOFs were prepared by the microemulsion method. A stock solution of starch (0.5 % wt) was made, also a certain amount of MoHis, MoPhe, or MoTry was dispersed in 10 mL of collagen solution (6 mg L^{-1}) to obtain MoMOF-collagen solution of 1.66% wt. The culture plates of 24 wells were used as molds for hydrogels, in each well was mixed 1 mL of MoMOF-collagen solution, 100 µL of starch solution, 20 µL of polyurethane, and 200 µL of phosphate-buffered saline solution (PBS-10X) to adjust the pH to 7, this step was made keeping the temperature from 4 to 5 ºC using an ice bath. Then, the reticulation was carried out by heating in a lab stove at 37ºC for 4 h to obtain the hydrogels, which were identified as CS-MoHis, CS-MoPhe and CS-Mary depending on the MOF employed.

2.4. ATR-FTIR and reticulation index

The chemical structure of materials was analyzed by ATR-FTIR using a Frontier, Perkin Elmer system, the spectra were recorded on dried hydrogel at 16 cm^{-1} of resolution, in a range from 4000 to 650 cm^{-1}, with an average of 16 scans. The crosslinking degree of composite hydrogels was analyzed by reacting the polymeric matrixes with ninhydrin (1 ml, 1 wt%, citrate buffer, pH 5.0) for 2 h at 90 °C. The absorbance of the liquid phase obtained after completing the reaction was measured by spectrophotometry at 567 nm (samples were prepared in triplicate), and the UV-Vis absorption measurements were performed with a ThermoScientific MultiSkan Sky spectrophotometer. Results were compared with unreticulated collagen, and the crosslinking degree was calculated with Equation (1) [14]:

$$Crosslinking\ degree, \% = \left(1 - \frac{A_{sample}}{A_{collagen}}\right) x100 \qquad (1)$$

Where Asample and Acollagen are the absorbances of solutions obtained after ninhydrin reacted with hydrogels and unreticulated collagen, respectively.

2.5. MTT essay and Cell migration

Red and green tomato seed cells were isolated and cultured in MS medium. The effect of the amino acid that contain the chemical structure of MoMOFs on the metabolic activity of vegetable cells growing in contact with hydrogels was evaluated by the MTT assay. For this, 1 mL of cell suspension (100 000 cells/mL) was seeded over hydrogels in polystyrene culture plates and incubated for 24 and 48 h at 37 °C (samples were prepared in triplicate). PBS-1X was mixed with 1 mL of cell suspension and it was used as the positive control. At the evaluation time (24 or 48 h), 15 μL of 3-(4,5-dimetilthiazol-2-yl)-2,5-diphenyltetrazolium) solution (1% wt. in sterilized PBS-1X) was added in each well and incubated for 2 h more. After that, 1 mL of propan-2-ol was added to dissolve the resulting blue formazan crystals. Aliquots of 200 μL were taken from the liquid medium and the absorbance was measured at 560 nm. Cell viability was calculated using Equation 2:

$$Cell\ viability, \% = \left(\frac{A_{sample}}{A_{control}}\right) x100 \qquad (2)$$

Where Asample and Acontrol are the absorbances of solutions obtained after MTT reacted with the sample and the control, respectively.

Cell migration micrographs were taken and evaluated test using a VELAB VE-403 inversed microscope. In this case, hydrogels were synthesized by the procedure mentioned above, however, after the collagen-MoMOFs solution was added to each well, 1 mL of vegetable cells was added, and then the other components such as starch, crosslinker and PBS-10X were added as mentioned before. After incubation at 37 ºC during 4 h, the hydrogels were dried to obtain the xerogels, which were observed using the microscope.

3. Results and Discussion

3.1. ATR-FTIR

The functional groups of CS-MoMOFs were analyzed by ATR-FTIR spectroscopy, the spectra are depicted in Figure 2.

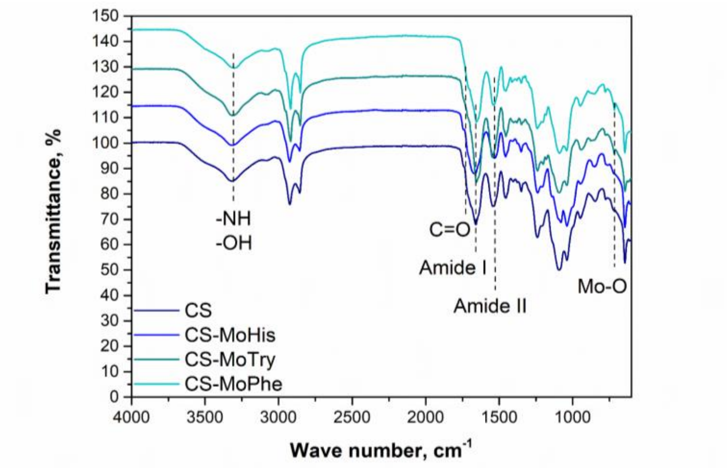

Figure 2. ATR-FTIR spectra of CS-MoMOFs

The absorption bands due to -OH and -NH vibrations are observed in the region of 4000 to 3200 cm^{-1}, indicating the amino groups and hydroxyl groups found in MoMOFs, collagen, and starch. The bands related to collagen are

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observed in 1633 and 1542 cm^{-1} and are assigned to the amide I and amide II which are typically found in the spectrum of polypeptides. The shoulder found in 1726 cm^{-1} is related to the C=O vibration of urea groups related to the crosslinking reaction between isocyanate groups of polyurethane and amino groups of collagen. While the absorption band at 734 cm^{-1} is attributed to Mo-O-Mo vibrations due to the molybdate cluster, indicating the incorporation of molybdenum from MoMOFs [8,11,15].

3.2. Reticulation index

Figure 3 shows the reticulation index results evidencing that in all cases the hydrogels present semi-interpenetrating networks (reticulation index lower than 60%), thus, the main interaction between collagen, starch, and MoMOFs are related to the hydrogen bridges related to the hydrophilic groups of each polymer, which are primarily amine and hydroxyl groups.

In general, the incorporation of MoMOFs increased the reticulation degree, being statistically equal the materials CS-MoHis and CS-MoTry. But the sample of CS-MoPhe had shown the maximum reticulation degree, this possibly obeys the more basic character of nitrogen in phenylalanine, which creates a more stable coordination bond between molybdenum and this amino acid. But also, the more hydrophobic nature of the phenolic groups generates a better dispersion of this MoMOF in the collagen-polyurethane-starch matrix, thus, favoring the reaction between the isocyanate groups of polyurethane with the primary amine groups of phenylalanine in MoPhe.

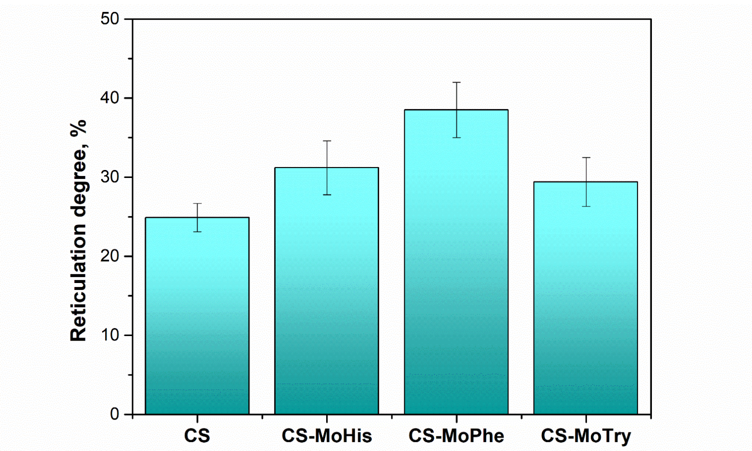

Figure 3. Reticulation degree of CS-MoMOFs

3.3. *In vitro* red tomato and green tomato cell viability

Figure 4(a) depicts the cell viability behavior of red tomato cells that proliferate over the surface of the hydrogels based on collagen-starch-MoMOFs. In all cases, the viability percentage is above 100% which is evidence of the non-cytotoxic effect of hydrogels. After comparing the results of 24 h with the Tukey test (α=0.05), it can be said that CS and CS-MoTry are statistically equal and showed major cell viability. While, after 48 h, the material CS-MoPhe and CS-MoTry were statistically equal and showed major cell viability. However, the increasing tendency of red tomato cell viability was found and maintained by the hydrogel CS-MoTry.

Figure 4(b) shows the cell viability percentage of green tomato cells over collagen-starch-MoMOFs hydrogels. The Tukey test (α=0.05) made to the results after 24 h indicates that CS-MoPhe and CS-MoTry are statistically equal

and showed major cell viability. When the Tukey tests are repeated to the results after 48 h, the green tomato cells are well-adapted, showing a decrease in cell viability, which is indicative of the decrease in cell metabolism and cellular respiration, provoking that cells are lethargic but with viability cells percentages major to 60%, being non-cytotoxic materials. Hence, after 48 h all materials have shown statistically the same cell viability.

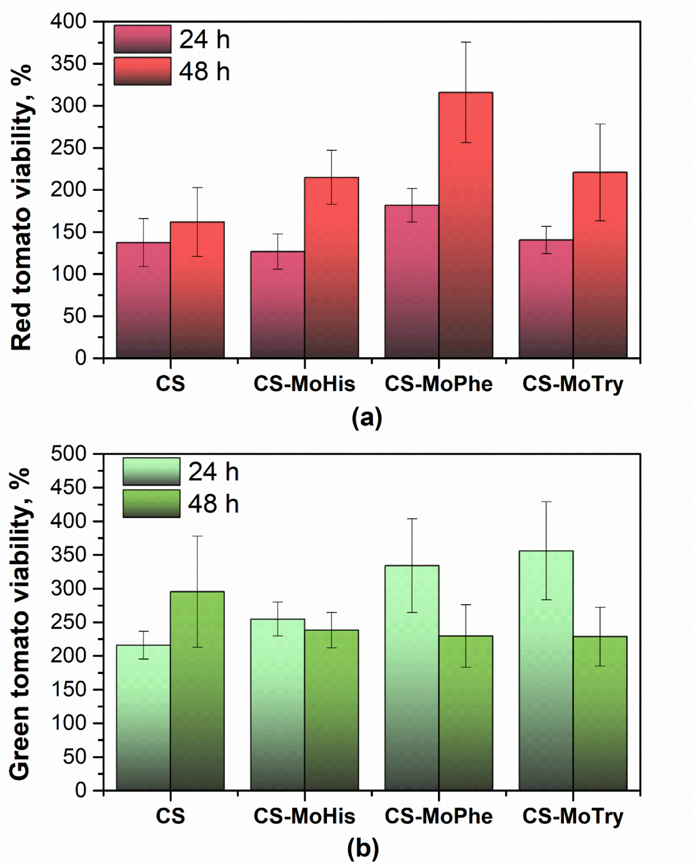

Figure 4. Red (a) and green (b) tomato cells viability living on CS-MoMOFs hydrogels

3.4. Cell migration

The micrographs of red and green tomato cells for cell migration tests are shown in Figure 5. In general, the presence of black spots evidences the presence of cells and thus, cell migration. It is observed that the more hydrophobic character of MoPhe avoids the cell migration of red and green tomato cells.

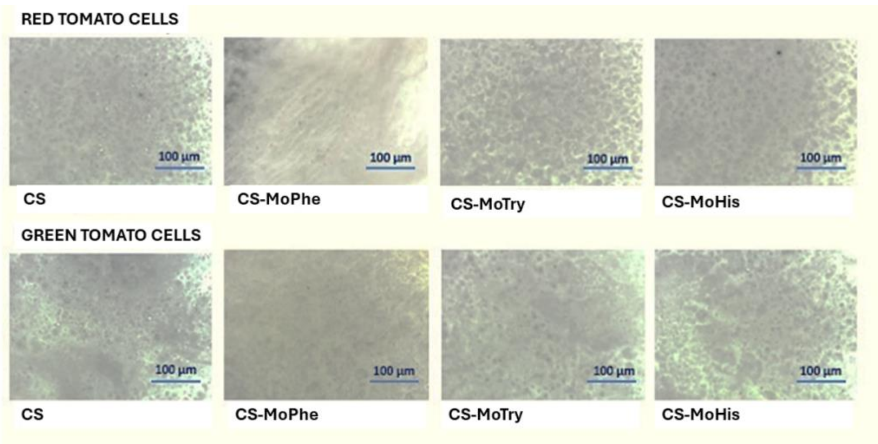

Figure 5. Red and green tomato cells into xerogels of CS-MoMOFs

While CS-MoTry and CS-MoHis allow cell migration similarly to the CS hydrogel, where scarcely black spots can be seen in CS-MoHis hydrogel, thus the more hydrophilic character of MoHis favors cell migration of red and green tomato cells.

4. Conclusions

The chemical structure of CS-MoMOFs indicates adequate interactions between collagen, polyurethane, starch, and Mo-MOFs, however, there is no displacement of the characteristic adsorption bands indicating physical interaction between the components. These results are in line with the reticulation degree that shows that these hydrogels form semi-interpenetrating networks by hydrogen bonding of the hydrophilic groups present in collagen, starch, and MoMOFs. The cell viability of red and green tomato cells indicates that Mo-Try is the best material to preserve the metabolism of vegetable cells during 24 and 48 h. In addition, it was found that the hydrophilic character of the amino acid facilitates cell migration, being the most promising materials CS-MoTry and CS-MoHis.

As a future work, it is suggested to proceed with a statical study of the germination of seeds from red and green tomatoes using as a substrate the composite hydrogels and a control (model earth). It is also recommended to make the *in vitro* biocompatibility of red and green tomato cells from leaves and stems living on these hydrogels. As well as, the evaluation of red and green tomato plant growth, measuring the amount of leaves and the plant height at a certain time. In this manner, the amino acid effect on the germination and growth of red and green tomato plants will be assessed.

Declarations

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Competing Interests Statement

The authors declare that they have no conflict of interest.

Consent for Publication

The authors declare that they consented to the publication of this study.

Authors' Contributions

All the authors took part in literature review, research, and manuscript writing equally.

Availability of data and material

Supplementary information is available from the authors upon reasonable request.

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